Title: LIGHT EMISSION ASSAY APPARATUS

Abstract: Analysis apparatus comprising a light detector (6) and at least two sample stations (10, 12) for receiving vessels (14) containing samples for analysis, the apparatus further comprising a mirror (28) which is movable between operative positions in which, respectively, the mirror (28) directs light emitted from samples at the respective sample stations (10, 12) to the light detector (6).
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments. For two-letter codes and other abbreviations, refer to the “Guidance Notes on Codes and Abbreviations” appearing at the beginning of each regular issue of the PCT Gazette.
LIGHT EMISSION ASSAY APPARATUS

This invention relates to light emission assay apparatus and is particularly, although not exclusively, concerned with apparatus for use in assay or analysis techniques which employ biological material, such as bacteria, which have a fluorescent or luminescent response which varies according to their ambient environment.

Some bacteria, such as the marine bacterium *Vibrio fischeri* are bioluminescent. That is, they emit light but the intensity of light emission varies in the presence of some chemicals, and in particular toxins. The light emission can, therefore, be used as a detectable signal which indicates the presence or absence of the chemical and also represents the concentration of the chemical in the environment, usually an aqueous solution, of the bacterium.

By way of example, such analysis techniques may be used to establish the concentration of toxins in samples of materials such as soil. Thus, a soil sample may be treated with a solvent in order to dissolve the target toxin to produce a test sample. A solution containing the bioluminescent organism is placed in a suitable container or cuvette, the test sample is added to the cuvette, possibly after further dilution of the test sample, so that the bioluminescent response of the organism can be monitored. In order to provide a reference, a control sample may be introduced to the same bioluminescent organism in a different cuvette, the control sample being identical to the test sample, except that it has had no contact with the soil.
The response of the organism, in terms of light emitted, is monitored. The monitoring period begins before the test sample and the control sample are placed in the cuvettes, so that the change in light emission can be detected. If the soil-derived test sample is toxic to the organism, its bioluminescence will be inhibited and this will appear as a difference in response between the organisms in the two cuvettes. By appropriate calibration, the magnitude of the difference can be used to derive the level of toxicity in the soil-derived sample.

Assaying apparatus, or luminometers, are known for conducting assays based on light emission. For example, US 5837195 discloses a luminometer comprising detector assemblies for detecting emitted light. A guide path is provided for cuvettes, and cuvettes are displaced along the guide path past dispensers for various reagents, past the detector assemblies, and past means for purging the cuvettes after analysis. This apparatus enables continuous sample analysis, but is a relatively complex and non-portable device which is not suitable for field testing of, for example, soil samples in conjunction with a control sample.

According to the present invention there is provided analysis apparatus comprising a light detector and at least two sample stations for receiving vessels for containing samples for analysis, the apparatus further comprising a mirror which is movable between operative positions in which, respectively, the mirror directs light emitted from samples at the respective sample stations to the light detector.
In this specification, the expression "mirror" is used in a broad sense to denote any device which reflects light, and this embraces devices other than polished or silvered surfaces, such as prisms.

The mirror may be mounted on a support body which is movable, for example in rotation, to move the mirror between the operative positions. The support body is preferably configured and positioned so that, when the mirror is in one of the operative positions to direct light from one of the sample stations to the light detector, the support body shields the other sample station, or one of the other sample stations, from the light detector. For this purpose, the support may be part-cylindrical, for example semi-cylindrical.

While apparatus in accordance with the present invention may have three or more sample stations, a preferred embodiment of the apparatus comprises two of the sample stations. Thus, one of the sample stations is used for a test sample and the other is used for a control sample. In the preferred embodiment, the mirror may be disposed between the two stations, and rotatable through 90° between the operative positions.

The mirror is preferably motor driven, and may be moved between the operative conditions in response to a computerised control system of the apparatus. The motor may be a stepping motor or may comprise a two position rotary solenoid.

The apparatus may comprise a carrier, for example in the form of a base plate, on which other components of
the apparatus, such as the light detector, the mirror, and components associated with the sample stations are mounted. The base plate may be provided with legs for supporting the apparatus on a suitable surface, so that the base plate is in a generally horizontal orientation. In this case, the mirror and the sample stations may be provided on the upper surface of the base plate, with the motor, if provided for the mirror, secured beneath the base plate.

The light detector may be mounted at or adjacent one edge of the base plate.

The present invention also provides a method of conducting analysis of two samples by detecting light emission from the samples, the method comprising alternately directing light from the samples to a light detector by means of a mirror which is movable between two operative positions.

For a better understanding of the present invention, and to show more clearly how it may be carried into effect, reference will now be made, by way of example, to the accompanying drawings, in which:

Figure 1 is a perspective view of analysis apparatus;

Figure 2 is a perspective view of the apparatus as viewed in a direction opposite that of Figure 1; and

Figure 3 is a plan view of the apparatus.

The apparatus shown in the Figures comprises a base plate 2 supported on legs 4. A light detector unit 6,
comprising a photo multiplier tube (PMT) is secured to the base plate 2 by a bracket 8. The bracket 8 has a window (not shown) for admitting light to the PMT of the unit 6 in operation of the apparatus. This window is disposed generally in the position indicated at W in Figure 3.

The base plate 2 supports two sample stations 10 and 12. Each sample station is configured to receive a respective cuvette 14 of square horizontal cross-section. The cuvettes 14 are made from transparent material such as glass or clear plastics. The cuvettes 14 are supported at the sample stations 10 and 12 on support blocks 22 disposed between respective side plates 16, 18. Spacers 20 extend between the side plates 16, 18. The units comprising the support blocks 22, the side plates 16 and 18 and the spacers 20 are secured to the base plate 2. The units are provided with Peltier heat pumps 23 which are secured to the support blocks 22, to maintain a desired temperature in the cuvettes 14. As can be appreciated from Figures 1 and 2, the side plates 18 have central apertures 24 which expose part of the adjacent face of the respective cuvette 14.

A support body 26 is situated between the sample stations 10 and 12, in the gap between the side plates 18. The support body is approximately semi-cylindrical with an upwardly extending longitudinal axis, assuming the base plate 2 is horizontal. The diametral face of the support body 26 carries a plane mirror 28. An electric stepping motor or a motor in the form of a two-position solenoid 30 is supported beneath the plate 2. Its output shaft (not shown) extends through an
opening in the base plate 2 and carries the support body 26.

The two operative positions of the stepping motor or of the solenoid 30 correspond to two operative positions of the support body 26 and the mirror 28. These positions are established accurately by means of an adjustable stop mechanism 31. One of these positions is shown in the Figures. To move to the other operative position, the support body 26 is rotated through \(90^\circ\) anticlockwise from the position shown in Figure 3. It will be appreciated from Figure 3 that light emitted from the cuvette 14 in the sample station 12 will pass through the window 24 and be reflected by the mirror 28 to the window \(W\) of the PMT in the light detector 6. In the other operative position, the mirror is positioned so that light emitted from the cuvette 14 at the sample station 10 will be directed by the mirror 28 to the window \(W\).

As shown in Figure 3, upright ribs 32 are provided on the side plates 14, these ribs abutting, or lying close to, the circular periphery of the support body 26. It will be appreciated that, in the operative position shown in Figure 3, the support body 26 abuts the ribs 32, so blocking the emission of light from the cuvette 14 in the sample station 10. Similarly, in the other operative position, cooperation between the support body 26 and the ribs 32 at the sample station 12 will block the emission of light from the cuvette 14 in the sample station 12.

Circuit boards 34 are secured to the side plates 16. These circuit boards carry lights in the form of light-
emitting diodes (LEDs) 36, of which the terminal pins 38 are visible in the Figures. The LEDs 36 are associated with means for measuring the turbidity of the liquid in the cuvettes 14.

The apparatus described may be used in an assay technique as described in our British Patent Application No (attorney's ref: HL83941), the disclosure of which is incorporated herein by reference. Consequently, details of the technique will not be described in detail in this specification, except as is necessary for understanding the present invention.

The apparatus may, for example, be used to test a soil sample for toxicity. The soil sample is treated so as to dissolve the toxins of interest, or to separate them from the soil particles. To achieve this, the soil sample is placed in a water-miscible solvent. The solvent, containing the toxin, may then be diluted and further ingredients may be added if required. The resulting solution constitutes a test sample.

A control sample is prepared using the same solvent, diluent and other ingredients in the same proportions as the test sample, except that the solvent had not been in contact with the soil sample and therefore does not contain the toxin.

A liquid containing a bioluminescent organism, for example Vibrio fischeri is introduced into each of the cuvettes 14. Such bacteria may be supplied and stored as freeze-dried or lyophilised cells, and can be reconstituted by adding a reconstitution solution of 2%
aqueous sodium chloride or phosphate buffer and allowing incubation at a temperature within a range of about 15°C to about 25°C. At this temperature, *Vibrio fischeri* will bioluminesce.

5 The cuvettes 14 are placed in the stations 10 and 12 and maintained at a desired constant temperature, for example 15°C by means of the Peltier heat pumps 23. The apparatus is then activated and the support body 26, with the mirror 28, switches between its two operative positions at regular intervals. These intervals may be of any practicable duration, but for most purposes they will be intervals in the range 0.1 to 10 seconds, and usually towards the upper part (ie 5 to 10 seconds) of this range. Thus, the light detector 6 receives, through the window W, light alternately from each of the cuvettes 14 in stations 10 and 12.

15 Since the organisms and their carrier solutions in the cuvettes 14 are substantially identical, the signals received (ie the intensity of light emitted by the light detector 6) should be approximately the same in both operative positions of the mirror 28.

20 When the signals from the bioluminescent organisms have stabilised, for example after approximately 5 to 7 minutes, the test sample prepared as described above is introduced into one of the cuvettes 14, for example that at the sample station 10, and the control sample is introduced to the other cuvette 14, for example that at the sample station 12. The presence of toxins in the test sample will inhibit the bioluminescence of the organism, and the reduced light output will be detected
by the light detector 6 when the mirror 28 is in the operative position.

Although the control sample contains no toxin, the increased dilution of the organism in the respective cuvette 14 and the effect of the chemicals in the control sample may also cause a reduction in bioluminescence. However, depending on the concentration and toxicity of the toxin in the test sample, the inhibition of bioluminescence will be different as between the test and control samples.

The apparatus is preferably linked to a computer which controls the operation of the apparatus, for example the movement of the mirror 28, and gathers data from the light detector 6 and processes that data to provide a useful output. By appropriate calibration, the computer may be programmed to output a reading representing the concentration of the toxin in the original soil sample.

If turbidity is detected in the samples, by means of the circuitry including the LEDs 36, appropriate steps are taken to compensate for the reduced light emission resulting from the turbidity.

The apparatus may include a GPS (Global Positioning System) module to enable the location of the apparatus, and consequently of the soil sample under analysis, to be automatically recorded. A GSM module (Global System for Mobile Communication) may also be included in the apparatus to enable remote downloading of data.
It will be appreciated that, since both the test sample and the control sample are prepared at substantially the same time and are analysed together by means of the movement of the mirror 28 between its operative positions, a single analysis sequence can, in a relatively short time; provide a useful output in a relatively simple manner. The apparatus is compact, compared with laboratory apparatus provided with automated conveying, dispensing and purging means, and can also be constructed in a relatively robust manner. The apparatus as disclosed is thus suitable for rapid analysis of soil and other samples in the field.

Although the present invention has been described with reference to bioluminescent organisms, it will be appreciated that the principles underlying the present invention can be applied to analysis techniques which rely on other forms of light generation, such as biofluorescence or chemical light generation.
CLAMS

1. Analysis apparatus comprising a light detector and at least two sample stations for receiving vessels containing samples for analysis, the apparatus further comprising a mirror which is movable between operative positions in which, respectively, the mirror directs light emitted from samples at the respective sample stations to the light detector.

2. Apparatus as claimed in claim 1, in which the mirror is mounted on a support body.

3. Apparatus as claimed in claim 2, in which the support body is configured so that, when in an operative position in which light emitted from a sample at one of the sample stations is directed by the mirror to the light detector, the support body blocks light emitted from a sample at the other sample station from reaching the light detector.

4. Apparatus as claimed in claim 2 or 3, in which the support body is substantially semi-cylindrical, the mirror being provided on a diametral surface of the support body.

5. Apparatus as claimed in any one of the preceding claims, in which there are two of the sample stations.

6. Apparatus as claimed in claim 5, in which the mirror is disposed between the sample stations.
7. Apparatus as claimed in any one of the preceding claims, in which the mirror is movable in rotation between the operative positions.

8. Apparatus as claimed in claim 7, in which the angle of displacement of the mirror between the operative positions is 90°.

9. Apparatus as claimed in any one of the preceding claims, in which the mirror is drivable between the operative positions by a motor.

10. Apparatus as claimed in claim 9, in which the motor comprises a two-position rotary solenoid.

11. Apparatus as claimed in claim 9, in which the motor is a stepping motor.

12. Apparatus as claimed in any one of the preceding claims, in which the sample stations and the mirror are supported on a base plate.

13. Apparatus as claimed in claim 12, in which the base plate is horizontal in normal operation of the apparatus, the sample stations and the mirror being provided on the upper face of the base plate.

14. Apparatus as claimed in claim 13, when appendant to claim 9, in which the motor is situated beneath the base plate.

15. Apparatus as claimed in any one of claims 12 to 14, in which the light detector is mounted at an edge of the base plate.
16. Analysis apparatus substantially as described herein with reference to, and as shown in, the accompanying drawings.

17. A method of conducting analysis of two samples by detecting light emission from the samples, the method comprising alternately directing light from the samples to a light detector by means of a mirror which is movable between two operative positions.

18. A method of conducting analysis of two samples, as claimed in claim 17 and substantially as described herein.
INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003828

A. CLASSIFICATION OF SUBJECT MATTER
IP: 7 G01N1/76

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>EP 0 515 129 A (ISRAEL AIRCRAFT IND LTD) 25 November 1992 (1992-11-25) * column 1, line 9 - column 3, line 19; column 5, line 50 - column 7, line 43; column 8, line 21 - column 9, line 30; figures 2 and 4</td>
<td>1-15,17</td>
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<td>A</td>
<td>GB 1 122 031 A (NAT RES DEV) 31 July 1968 (1968-07-31) * page 4, lines 10-13 *</td>
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Further documents are listed in the continuation of box C. X Patent family members are listed in annex.

* Special categories of cited documents:
* A* document defining the general state of the art which is not considered to be of particular relevance
* S* earlier document but published on or after the international filing date
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another document, or otherwise special reason (as specified)
* O* document referring to an oral disclosure, use, exhibition or other means
* P* document published prior to the international filing data but later than the priority data claimed

*"* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*:"* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered not to involve an inventive step when the document is taken alone
*X:* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is taken alone
*Y:* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
*:"* document member of the same patent family

Date of the actual completion of the international search 6 August 2004
Date of mailing of the international search report 13/08/2004

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Hoogen, R

Form PCT/ISA/210 (second sheet) [January 2004]
Continuation of Box II.2

Claims Nos.: 16, 18

In claims 16 and 18 the attempt is made to define the claimed subject-matter by reference to the drawings and the description, respectively. It is not clear which combination of features shall be thereby specified.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
**INTERNATIONAL SEARCH REPORT**

**Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☑ Claims Nos.: 16, 18 because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   
   see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
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<th>Publication date</th>
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<tr>
<td>GB 1122031</td>
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